IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Matsuzaki et al.	
	Art Unit: 1656
Application No.: 10/790,224	Examiner: Steadman
Filing Date: March 2, 2004	Attorney Ref. No.: US-162
For: METHOD FOR PRODUCING L-ARGININE OR L-LYSINE BY FERMENTATION	

DECLARATION UNDER 37 C.F.R. § 1.132

We, Yumi Matsuzaki, Jun Nakamura, and Kenichi Hashiguchi, declare as follows:

1. We are employees of Ajinomoto Co., Inc. located at 1-1 Suzuki-shi, Kawasaki-ku, Kawasaki-shi, Kanagawa, Japan, which is engaged in the business of research and development of fermentation processes and products, among other things.

2. We are co-inventors of the above-identified patent application.

3. We have conducted the experiments described herein and present them as evidence in the above-identified patent application for consideration by the Examiner in support of our Amendment and supporting arguments filed January 5, 2007.

4. The data described herein shows that GS activity is very low in L-glutamine-producing strains, and therefore enhancement of GS activity is not a critical factor in amino acid fermentation.

5. Activities of GS and GDH in a sulfaguanidine-resistant mutant:

A wild-type strain of *Brevibacterium flavum* ATCC 14067 (Strain A) and a sulfaguanidine-resistant strain derived from Strain A (Strain B) were inoculated into a seed medium (30 g/L glucose, 1.5 g/L KH_2PO_4, 0.01 g/L $MgSO_4 \cdot 7H_2O$, 100 μg/L VB_1-HCl, 3

μg/L biotin, 200 mg/L soybean hydrolysates, 1.5 g/L urea, and 0.02 ml/L GD-113, at pH=6.8 (NaOH)), and cultured overnight at 31.5°C with shaking. Then GS activity and GDH activity were measured as follows.

6. Measurement of GS activity:

The above-described culture of Strain A and Strain B were centrifuged (10,000rpm for 1min.) and the collected cells were washed twice with 100mM imidazole-HCl (pH=7.0). The cells were then disrupted by sonication (COSMO BIO Bioruptor) followed by centrifugation (10,000rpm for 15min.) to remove cellular debris. The resulting supernatant was ultracentrifuged (10,000rpm for 15min.) to remove the membrane fraction. Thus, a crude enzyme solution was obtained. The GS activity of the crude enzyme solution was measured in the same manner as the method described in the specification of the present application (see Example 2(2)).

7. Measurement of GDH activity:

The above-described culture of Strain A and Strain B were centrifuged (10,000rpm for 1min.) and the collected cells were washed twice with 50mM Tris-HCl (pH=7.5). The cells were then disrupted by sonication (COSMO Bio Biorupter), followed by centrifugation (10,000 rpm for 15min.) to remove cellular debris. Thus, a crude enzyme solution was obtained. The GDH activity of the crude enzyme solution was measured in the same manner as the method in the specification of the present application (see Example 4(3)).

8. Results:

The results of the measurement of GS and GDH activities are shown in Table 1.

Table 1

Enzyme activity	ATCC14067 (Strain A)	Sulfaguaniine resistant mutant (Strain B)
GS (U/mg)	0.034	0.033
GDH (U/mg)	1.65	1.96

9. We further declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By: _____
Yumi Matsuzaki

By: _____
Jun Nakamura

By: _____
Kenichi Hashiguchi

Date: January 8, 2007